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Collagen Turnover in Wound Repair—A Macrophage Connection

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In this issue, Rohani *et al.* (2015) report on the role of macrophage-derived stromelysin-2 (matrix metalloproteinase (MMP)-10) in promoting the turnover of extracellular matrix (ECM) during cutaneous wound repair. They provide evidence that MMP-10 specifically enhances collagenolytic activity of murine MMP-13 produced by M2-like macrophages. These results emphasize the important role of macrophage-derived MMP-10 in regulating tissue remodeling and scar formation during wound healing.

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Proteolysis in wound healing

Wound healing in the skin is a complex and a strictly controlled process that involves interplay between different cell types. Cutaneous wound repair consists of temporally overlapping, but functionally and histologically distinct, phases—i.e., hemostasis, an inflammatory response, re-epithelization, granulation tissue formation, and tissue remodeling—all of which require precise communication among the various cell types. The resolution of inflammation is an important event in normal wound repair, and failure in this results in persistent inflammation and impaired wound closure (Eming *et al.*, 2007). Subsequently, activation and differentiation of fibroblasts to collagen-producing, α-smooth muscle actin-positive myofibroblasts result in the formation of collagen-rich granulation tissue, which provides tensile strength to the healing wound. Finally, wound contraction and remodeling and resolution of the granulation tissue ECM by proteolysis and endocytosis take place, resulting in the restoration of structure and function of the skin, with minimal scar formation (Toriseva and Kähäri, 2009). Failure in this controlled turnover of deposited ECM may result in excessive scar formation.

MMPs are among the major proteolytic enzymes produced and activated by a variety of cells participating in cutaneous wound repair. MMPs are extracellular endopeptidases, which can be classified into distinct subgroups according to their substrate specificity. In intact skin, the production of MMPs is in general low, but injury to the skin results in a rapid increase in the production of MMPs. Accordingly, several MMPs are expressed in adult human wound tissue, including collagenase-1 (MMP-1), collagenase-2 (MMP-8), gelatinase-A (MMP-2), gelatinase-B (MMP-9), membrane type 1 MMP (MMP-14), MMP-19, MMP-26, MMP-28, stromelysin-1 (MMP-3), and stromelysin-2 (MMP-10). MMPs can cleave ECM components, releasing biologically active peptide fragments. In addition, they are able to process several nonmatrix substrates—e.g., growth factors embedded in the ECM or growth factor receptors on cell surfaces (Nissinen and Kähäri, 2014). Furthermore, MMPs have an important role in tissue repair by promoting the resolution of the granulation tissue and collagenous scar. However, the functions of specific MMPs in wound healing and tissue fibrosis are not fully understood.

In this issue, Rohani *et al.* (2015) have examined the role of macrophage-derived MMP-10 during cutaneous wound healing in a mouse model. Previously, the expression of MMP-10 had been documented to occur in migrating keratinocytes at the leading edge of human and mouse cutaneous wounds (Saarialho-Kere *et al.*, 1992; Rechardt *et al.*, 2000). In addition, expression of active mutant of MMP-10 in keratinocytes of transgenic mice resulted in impaired organization of the wound epithelium, thus providing mechanistic evidence for the role of MMP-10 in keratinocyte migration (Krampert *et al.*, 2004).

Collagenases in wound repair

Collagenases (MMP-1, -8, and -13) are the principal secreted proteinases capable of cleaving native fibrillar collagens of types I, II, III, V, and IX. Two mouse counterparts of human MMP-1 have been identified, Mcol1-A and Mcol1-B, but only Mcol1-A can cleave fibrillar collagens (Balbín *et al.*, 2001). Murine MMP-13 shows the highest homology with human MMP-13. However, murine MMP-13 and human MMP-1 are expressed in similar settings, including wound repair, indicating that mouse MMP-13 is a functional homolog of human MMP-1.

Collagenases identified in normally healing human cutaneous wounds are MMP-1 and MMP-8 (Saarialho-Kere *et al.*, 1992; Inoue *et al.*, 1995; Nwomeh *et al.*, 1999). MMP-1 is expressed by migrating keratinocytes at the edge of human wounds, and the activity of MMP-1 is required for migration of keratinocytes on native type I collagen (Saarialho-Kere *et al.*, 1992; Pilcher *et al.*, 1997). Notably, MMP-1 is expressed by same cells in wound edges as is MMP-10 (Saarialho-Kere *et al.*, 1994). MMP-1 is also expressed by fibroblasts in granulation tissue during wound healing, and it is involved in remodeling of wound collagen. MMP-8 is mainly stored in secretory granules of neutrophils and released upon activation of these cells. Mice lacking MMP-8 show impaired re-epithelization and delayed wound healing, associated with persistent inflammation and a lag in neutrophil infiltration (Gutiérrez-

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Clinical Implications

- Elucidation of macrophage function and regulation during tissue repair at a molecular level is important for understanding the pathogenesis of tissue fibrosis.
- The findings of Rohani *et al.* (2015), showing that macrophage-derived MMP-10 controls the clearance of ECM during the repair of cutaneous wound may help in developing new therapeutic modalities to tissue fibrosis.
- It is possible that targeted regulation of MMP-10 activity could promote the clearance of excessive ECM and this way be of therapeutic benefit in tissue fibrosis.

Fernández *et al.*, 2007). Adenoviral delivery of MMP-8 gene into the liver has been shown to reverse tissue fibrosis in a rat model of liver cirrhosis, suggesting a role for MMP-8 in resolution of tissue fibrosis (Siller-López *et al.*, 2004).

In murine skin wounds, mouse MMP-13 shows a similar expression pattern as does MMP-1 in human wounds. However, expression of MMP-13 is not detected in normally healing adult human wounds, but in chronic cutaneous ulcers the expression of MMP-13 by fibroblasts has been documented (Vaalamo *et al.*, 1997). On the other hand, MMP-13 is expressed by fibroblasts in fetal skin and gingival wounds, two types of wounds that have been characterized by scarless wound repair (Ravanti *et al.*, 1999, 2001). Studies with MMP-13-null mice have shown that murine MMP-13 has a role in keratinocyte migration, vascularization, granulation tissue formation, and wound contraction (Hattori *et al.*, 2009; Toriseva *et al.*, 2012). Evidence for the anti-fibrotic role of MMP-13 has been studied in rodent models of tissue fibrosis. Studies with MMP-13-null mice suggest that MMP-13 exhibits anti-fibrotic properties in murine hepatic fibrosis (Fallowfield *et al.*, 2007). In addition, adenoviral delivery of MMP-13 has been shown to promoting resolution of hepatic fibrosis (Endo *et al.*, 2011).

Macrophages in wound healing

Following cutaneous wounding, circulating monocytes migrate to the site of injury and differentiate into macrophages. In general, macrophages have a role in all phases of wound healing, and depletion of macrophages results in delayed wound repair, reduced granulation tissue formation, and delayed re-epithelialization and scar deposition (Lucas *et al.*, 2010). Functional phenotypes of macrophages in injured skin can be divided into two distinct subgroups—i.e., the classically activated M1 subtype and alternatively activated M2 macrophages (Mantovani *et al.*, 2002). Several mediators, such as bacterial lipopolysaccharide, and inflammatory cytokines can stimulate macrophages to differentiate into M1 macrophages that include antimicrobial properties (Mosser and Edwards, 2008). IL-4 and IL-13 induce differentiation to M2 macrophages, which have an important role in the later phase of wound healing when clearance of ECM and new tissue formation take place (Mahdavian Delavary *et al.*, 2011). These cytokines also induce the expression of MMP-13 in macrophages. Furthermore, upon phagocytosis of apoptotic cells, M1 macrophages may revert to M2 macrophages and contribute to the resolution of inflammation by clearing senescent cells and cellular debris (Mahdavian Delavary *et al.*, 2011). M2 macrophages also produce transforming growth factor-β, and, they may in this way promote various aspects of wound healing, such as fibroblast chemotaxis, angiogenesis, ECM deposition, and wound contraction. Studies with macrophage-depleted mice also demonstrate impaired turnover of granulation tissue, resulting in scar formation (Brancato and Albina, 2011; Mahdavian Delavary *et al.*, 2011).

In this issue, Rohani *et al.* (2015) report increased scar formation in wounded skin of MMP-10-null mice due to reduced collagenolytic activity. Furthermore, their data show that the number of macrophages was not decreased nor was the migration rate of macrophages affected in the MMP-10-deficient mice. However, in the wounds of MMP-10-null mice, the production and activity of MMP-13 produced by macrophages was reduced, resulting in impaired scar resolution in the wounds. These results provide novel mechanistic evidence for an important role for MMP-10 in regulating the collagenolytic activity of alternatively activated M2 macrophages that are responsible for scar resolution during cutaneous wound repair.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Playing Polo-Like Kinase in NRAS-Mutant Melanoma

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NRAS-mutant melanomas are extremely aggressive and highly resistant to currently available therapeutic modalities. Hence, new targets and therapeutic strategies for NRAS-driven melanomas are needed. As blocking NRAS directly has not been possible thus far, targeting downstream NRAS effectors, such as MAPK/ERK kinase (MEK), is being evaluated as an alternative therapeutic approach. However, blocking this pathway alone has limited efficacy. In this issue, Posch *et al.* report on a combination approach co-targeting polo-like kinase 1 and MEK in NRAS-mutant melanomas. This combination triggers a dual blockade of the cell cycle machinery, leading to apoptosis, and providing a new strategy to treat NRAS-mutant melanoma.

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Mutant-NRAS in melanoma

RAS is mutated in approximately 30% of human cancers. For example, neoplasms of the skin, pancreas, and urinary tract carry activating mutations in the RAS isoforms NRAS, KRAS, and HRAS, respectively (Prior *et al.*, 2012). In melanoma, approximately 25% of tumors harbor NRAS mutations. Most NRAS mutations affect codon 61, locking the small G protein in the active guanosine triphosphate-bound form and leading to persistent RAS signaling (Ascierto *et al.*, 2013; Burd *et al.*, 2014). Oncogenic NRAS activates several signaling pathways, including the mitogen-activating protein kinase (MAPK), PI3K/mTOR, and Ral GDP dissociation stimulator (RalGDS), resulting in aberrant cell proliferation and increased tumor cell survival. Attempts to inhibit oncogenic NRAS directly have not been successful to date, prompting a search for alternative strategies to blunt NRAS signaling. Suppression of the NRAS effector pathway MAPK (RAF/MEK/ERK) with MEK (MAPK/ERK kinase) inhibitors (MEKi) has been evaluated in clinical trials of NRAS-mutant melanoma; however, response rates were barely 20% and short-lived (Ascierto *et al.*, 2013). Immune checkpoint inhibitors are only approved for BRAF-mutant patients thus far, and their role in treating NRAS-mutant melanoma remains to be established. Additionally, it has been reported recently that NRAS-mutant melanomas are associated with lower levels of lymphocyte infiltration, suggesting a more immunosuppressive microenvironment and possibly poor responses to immunotherapy (Thomas *et al.*, 2015). Consequently, identifying novel targets and/or co-targeting other NRAS-specific vulnerabilities are essential for designing effective treatments for these types of tumors. In this issue of the *Journal of Investigative Dermatology*, Posch *et al.* (2015) report on suppressing a mitotic master Ser/Thr kinase, polo-like kinase 1 (PLK1) in combination with MEK inhibition as a therapeutic strategy for NRAS-mutant melanoma (Figure 1; Posch *et al.*, 2015).

Polo-like kinase: a new therapeutic target for NRAS-mutant melanoma

PLK1 has emerged as a therapeutic target in cancer as it regulates cell cycle

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